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**(54) Title:** GENE EXPRESSION CASSETTE CONTAINING NON-CODING SEQUENCE OF GROWTH HORMONE GENE

**(57) Abstract**

The present invention provides a genetic expression cassette for use in obtaining expression of a cDNA sequence in animal cells. The expression cassette comprises an inducible promoter and the 3′ non-coding sequence of exon 5 of the growth hormone gene or a portion thereof. The cDNA sequence is inserted between the inducible promoter and the exon 5 of the growth hormone genes.

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# GENE EXPRESSION CASSETTE CONTAINING NON-CODING SEQUENCE OF GROWTH HORMONE GENE

## FIELD OF THE INVENTION

The present invention relates to a gene expression cassette which enables expression of cDNA sequences in animal cells. The expression cassette of the present invention is particularly useful in achieving high-level expression of bacterial and/or plant genes in animal cells.

## BACKGROUND OF THE INVENTION

It is now possible to transfer unique pieces of DNA between organisms in such a way that the transferred material becomes a functional part of the genetic information of the recipient organisms. The animals that are produced by this technique are termed "transgenic". One application of this technology is to transfer biochemical pathways from bacteria to domestic animals in order to increase animal productivity. One difficulty which is frequently encountered in efforts to produce such transgenic animals is the lack, or very low levels of expression of the transferred DNA sequences.

The present inventors have developed a genetic expression cassette which provides information for the expression of heterologous genes, in particular bacterial genes, in mammalian cells and in several tissues of transgenic animals, at levels that provide ready detection of the encoded polypeptides.

The expression cassette consists of two components:- a regulatory element and a non-coding sequence from the growth hormone gene.

## SUMMARY OF THE PRESENT INVENTION

Accordingly, in a first aspect the present invention consists in a genetic expression cassette for use in obtaining expression of a cDNA sequence in animal cells, the cassette comprising an inducible promoter and the 3' non-coding sequence of exon 5 of the growth hormone gene or a portion thereof, the cDNA sequence being positioned

between the inducible promoter and the 3' non-coding sequence of exon 5 of the growth hormone gene.

In a preferred embodiment of the present invention the inducible promoter is the immediate upstream nucleotide sequence of the sheep metallothionein-Ia gene.

The expression cassette of the present invention provides a means for the expression of a wide range of genes in transgenic animals, including the coding sequences of bacterial enzymes, plant chitinases, insecticidal scorpion venom toxin and the insecticidal protein of the bacteria <u>Bacillus thuringiensis</u>. In a preferred embodiment of the present invention the cDNA sequence is selected from the group consisting of <u>cysE</u>, <u>cysK</u>, <u>aceA</u> and <u>aceB</u> genes of <u>Escherichia coli</u> and the coding sequences of plant chitinases.

In yet a further preferred embodiment of the present invention the genetic expression cassette has a sequence substantially as shown in Figure 1.

The expression cassette of the present invention is useful in obtaining high levels of expression of cDNA sequences in animal cells. Accordingly, in a second aspect the present invention consists in a non-human animal including the genetic expression cassette of the first aspect of the present invention.

In a preferred embodiment of this aspect the animal is ovine or bovine.

## DETAILED DESCRIPTION OF THE INVENTION

In order that the nature of the present invention may be more clearly understood, preferred forms thereof will now be described with reference to the following examples and figures in which:-

Figure 1 shows the nucleotide sequence of the expression cassette of the present invention;

Figure 2 shows the sequence of MTCE10;

Figure 3 shows the sequence of MTCK7;

Figure 4 shows the sequence of MTCEK1;
Figure 5 shows the sequence of MTAceA2;
Figure 6 shows the sequence of MTAceB2;
Figure 7 shows the sequence of MTAceAB11; and
Figure 8 shows levels of radiolabelled cysteine in transgenic mice containing MTCEK1 (———) and in control mice (- - - -). The arrow shows the position of cysteic acid.

Initially, a number of gene arrangements for expression of the cysK gene in murine L-cells were trialled. The trialled constructs were as follows:-

pMTCK7 - sheep metallothionein-Ia gene promoter - cysK - exon 5 of sheep growth hormone.

pMTCK8 - sheep metallothionein-Ia promoter - exon 1 sheep growth hormone - cysK - exon 5 sheep growth hormone.

pMTCK11 - sheep metallothionein-Ia promoter - cysK - whole sheep growth hormone.

pMTCK12 - sheep metallothionein-Ia - exon 1 sheep growth hormone - cysK - exons 2, 3, 4 and 5 sheep growth hormone.

The constructs were transfected into murine L-cells and the O-acetylserine sulfhydrylase activity of the transfected cells measured. The results obtained are set out in Table 1.

TABLE 1

O-Acetylserine Sulfhydrylase Activity in Transfected Murine L-Cells Using Various cysK Genes

| Gene | Enzyme Activity (nMoles cysteine produced/mg protein/30 min) |
|---|---|
| pMTCK7 | 1350 ± 24 |
| pMTCK8 | 510 ± 13 |
| pMTCK11 | 162 ± 17 |
| pMTCK12 | 159 ± 6 |

(values represent the means of two determinations)

As can seen from these results exon 5 of the growth hormone gene of sheep is required for optimum expression of genes inserted into the cassette. Other combinations which comprise larger portions of the sheep growth hormone gene are less effective in providing expression.

Two examples of the function of the expression cassette are shown as follows:

1. <u>Expression of the cysE and cysK genes of E. coli in transgenic animals</u>

In order to provide a pathway for the biosynthesis of the amino acid cysteine, the coding sequences for the bacterial enzymes serine transacetylase and O-acetylserine sulfhydrylase have been inserted into the expression cassette.

Three genes are described. Genes 1 and 2 each encode single bacterial proteins, gene 1 encoding the protein serine transacetylase and gene 2 encoding the protein O-acetylserine sulfhydrylase. Gene 3 is a compound gene constructed from gene 1 and gene 2, and encodes both the serine transacetylase protein and the O-acetylserine sulfhydrylase protein.

The expression cassette of the present invention was produced using methods well known in the art. Briefly this involves the steps of:

1. Isolation and cloning of the sheep metallothionein-Ia promoter sequence.

2. Isolation and modification of the bacterial coding sequence and fusion to the bacterial coding sequence.

3. Fusion of exon 5 of the sheep growth hormone gene to the metallothionein promoter/bacterial coding sequence complex.

In order to provide further details on construction of the cassette the procedure followed in construction of MTCE10 was as follows:

Step 1.

A bacterial plasmid containing the sheep metallothionein-Ia gene was digested with the restriction enzymes Eco RI and BamH1 and a DNA fragment encoding the promoter region of the gene separated by agarose gel electrophoresis and cloned in the plasmid vector pUC8.

Step 2.

The coding sequence and associated 5' and 3' DNA encompassing the cysE gene of Escherichia coil was cloned in the plasmid vector pGEM3 as an Eco R1 fragment excised from a lambda transducing phage containing portion of the E.coil chromosome. Sub-fragments of this insert were then cloned into the bacteriophage M13 and the clones encompassing the bacterial initiation codon and the bacterial stop codon were used for site-directed mutagenesis to introduce a Bam H1 site at the 5' end of the coding sequence and a Sau 3A site at the 3' end of the gene. The mutagenesis was carried out on single-strand DNA by conventional procedures and the resulting modified DNA used to replace the corresponding DNA fragments in the insert of the original pGEM3 clone. A Bam H1 - Sau 3A fragment of DNA was then excised from this plasmid and inserted into a similarly digested sample of the plasmid containing the metallothionein-Ia sequence.

Step 3.

The plasmid containing the metallothionein-Ia promoter-csyE coding sequence was digested with Pvu II (adjacent to the introduced Sau 3A site) and to this was ligated a blunt-ended Pst 1 DNA fragment isolated from the sheep growth hormone gene and encompassing exon 5. Plasmids containing the correct orientation of the growth hormone sequence were identified by restriction enzyme mapping.

GENE DETAILS

Gene 1 (MTCE10)

This gene consists of the sheep metallothionein-Ia gene promoter sequence joined to the coding sequence of the Escherichia coli cysE gene at a unique BamH1 restriction enzyme site. This sequence was then joined to the 3' sequence of exon 5 of the sheep growth hormone gene. Minor sequence modification in the vicinity of the initiation and stop codons of the bacterial cysE gene were made by site-directed mutagenesis using synthetic oligonucleotides. The metallothionein promoter replaces all regulatory sequences located 5' to the cysE gene coding sequence, and the growth hormone exon 5 sequence replaces all untranslated sequences located 3' to the cysE gene coding sequence. The gene is approximately 3580 base pairs in length, of which 2827 nucleotides have been sequenced. The sequence of gene 1 is shown in Figure 2.

Gene 2 (MTCK7)

This gene consists of the sheep metallothionein-Ia gene promoter sequence joined to the coding sequence of the Escherichia coli cysK gene at a unique Sal 1 restriction enzyme site. This sequence was then joined to the 3' sequence of exon 5 of the sheep growth hormone gene. Minor sequence modification of the cysK gene in the vicinity of the initiation codon was made by site-directed mutagenesis using a synthetic oligonucleotide. The metallothionein promoter replaces all regulatory sequences located 5' to the cysK coding sequence, and the sheep growth hormone exon 5 replaces all untranslated sequence located 3' to the cysK coding sequence. The size of the gene is approximately 3750 base pairs in length, of which 2957 base pairs have been sequenced. The sequence of gene 2 is shown in Figure 3.

Gene 3 (MTCEK1)

This gene consists of a fusion of genes 1 and 2 to

create a single DNA sequence that encodes both the serine transacetylase and the O-acetylserine sulfhydrylase enzymes. Each coding sequence is separately regulated by its own adjacent sheep metallothionein-Ia gene promoter sequence, and each coding sequence is separately followed by the 3' sequence of exon 5 of the sheep growth hormone gene. The gene is approximately 7550 base pairs in size, of which 5784 nucleotides have been sequenced. The sequence of gene 3 is shown in Figure 4.

Example 2. The expression of the glyoxylate cycle in transgenic animals

In order to provide the enzymes needed for the operation of the glyoxylate cycle in transgenic animals, the *E. coli* genes encoding the enzymes isocitrate lyase and malate synthase have been inserted into the expression cassette.

Three genes are described. Genes 1 and 2 each encode single bacterial proteins, gene 1 encoding the protein isocitrate lyase and gene 2 encoding the protein malate synthase. Gene 3 is a compound gene constructed from gene 1 and gene 2, and encodes both the isocitrate lyase and the malate synthase proteins.

GENE DETAILS

**Gene 4 (MTAceA2)**

This gene consists of the sheep metallothionein-Ia gene promoter sequence joined to the coding sequence of the *Escherichia coli* *aceA* gene at a unique BamH1 restriction enzyme site. This sequence was then joined to the 3' sequence of exon 5 of the sheep growth hormone gene. Minor sequence modification in the vicinity of the initiation and stop codons of the bacterial *aceA* gene were made by site-directed mutagenesis using synthetic oligonucleotides. The metallothionein promoter replaces all regulatory sequences located 5' to the *aceA* gene coding sequence, and the growth hormone exon 5 sequence

replaces all untranslated sequences located 3' to the aceA gene coding sequence. The gene is approximately 3580 base pairs in length, of which 2827 nucleotides have been sequenced. The sequence of gene 4 is shown in Figure 5.

Gene 5 (MTAceB2)

This gene consists of the sheep metallothionein-Ia gene promoter sequence joined to the coding sequence of the *Escherichia coli* aceB gene at a unique Sal 1 restriction enzyme site. This sequence was then joined to the 3' sequence of exon 5 of the sheep growth hormone gene. Minor sequence modification of the aceB gene in the vicinity of the initiation codon was made by site-directed mutagenesis using a synthetic oligonucleotide. The metallothionein promoter replaces all regulatory sequences located 5' to the aceB coding sequence, and the sheep growth hormone exon 5 sequence replaces all untranslated sequence located 3' to the aceB coding sequence. The size of the gene is approximately 3750 base pairs in length, of which 2957 base pairs have been sequenced. The sequence of gene 5 is shown in figure 6.

Gene 6 (MTAceAB1)

This gene consists of a fusion of genes 1 and 2 to create a single DNA sequence that encodes both the isocitrate lyase and the malate synthase enzymes. Each coding sequence is separately regulated by its own adjacent sheep metallothionein-Ia gene promoter sequence, and each coding sequence is separately followed by the 3' sequence of exon 5 of the sheep growth hormone gene. The gene is approximately 7550 base pairs in size, of which 5784 nucleotides have been sequenced. The sequence of gene 6 is shown in Figure 7.

REGULATION OF THE GENES

Regulation in Cultured Cells

Genes 1 to 6 have been transfected into mouse L-cells

in culture to produce stably transformed cell lines. The expression of each gene was measured by:

1. Northern blot analysis of extracted RNA.
2. Enzyme assay of cell extracts.

An RNA transcript of the expected size was detected in RNA extracted from each cell line, using a probe specific for the appropriate coding sequence of each gene. The intensity of the hybridisation increased when cells were grown in a medium containing 10 uM zinc sulphate, indicating that the genes were regulated by heavy metals.

The results of enzyme assays of cell extracts from each of the transformed cell lines are shown in Table 1 (genes 1 - 3) and Table 4 (genes 4,5). High levels of activity of serine transacetylase, O-acetylserine sulfhydrylase, isocitrate lyase and malate synthase were measured in the appropriate cell extracts, and the enzyme levels were increased when cells were grown in zinc-supplemented growth media.

Cell extracts prepared from cells containing the fusion gene MTCEK1 contained both serine transacetylase and O-acetylserine sulfhydrylase enzyme activities, indicating that both coding sequences within the fusion gene were transcribed and translated. Furthermore, when extracts from this cell line were incubated with the substrates serine and $H_2S$, substantial quantities of cysteine were produced, evidence that the entire biochemical pathway is operational in these cells. Similarly, cell extracts prepared from the cells containing the fusion gene MTAceAB1 contained both isocitrate lyase and malate synthase enzyme activities, indicating that both coding sequences within the fusion gene were transcribed and translated.

**Expression in Transgenic Mice**

Genes 1 to 6 were each transferred to transgenic mice

by the technique of single-cell embryo pronuclear microinjection. Mice containing the new genes were analyzed for expression by extracting mRNA and preparing cell-free supernatants from various tissues including liver, kidney and intestine. As shown in Tables 3 and 5, high levels of activity of the various enzymes were detected in appropriate transgenic mice. Furthermore, the expression of the genes in the intestinal tissues was highly zinc-dependent.

TABLE 2

Expression of MTCE10 and MTCK7 in transformed mouse L-cells

| | Serine Transacetylase | | O-acetylserine Sulfhydrylase | |
|---|---|---|---|---|
| cells | -Zn | +Zn | -Zn | +Zn |
| control | 0 | 0 | 0 | 0 |
| MTCE10 | 1281 | 2706 | - | - |
| MTCK7 | - | - | 38 | 1367 |
| MTCEK1 | 120 | 360 | 1082 | 7790 |

Values are nmoles product formed/mg protein/30 min

TABLE 3

Activity of serine transacetylase (SAT) and O-acetylserine sulphydrylase (OAS) in tissue extracts prepared from transgenic mice. CK7-26 contains the gene pMTCK7, CE10-29 contains pMTCE10 and CEK1-28 and CEK1-8 contains pMTCEK1. Specific activity is measured as nmoles substrate utilised (SAT) or product formed (OAS/30 min/mg protein.

| MOUSE LINE | ORGAN | SAT | OAS |
|---|---|---|---|
| CK7-26 | Intestine | - | 206 |
| | Kidney | - | 352 |
| | Liver | - | 13 |
| CE10-29 | Intestine | 6,546 | - |
| | Kidney | 0 | - |
| | Liver | 0 | - |
| CEK1-28 | Intestine | 1,161 | 2,797 |
| | Kidney | 0 | 24 |
| | Liver | 0 | 3 |
| | Brain | 16 | 86 |
| CEK1-8 | Intestine | 4,522 | 12,778 |
| | Kidney | 105 | 128 |
| | Liver | 9 | 3 |
| | Brain | 0 | 245 |
| | | 0 | 158 |
| | Skin | 0 | 329 |
| | | 6 | 295 |

In order to assess the ability of transgenic mice containing the pMTCEK1 gene to produce cysteine, transgenic mice including this gene and control mice were given 25 mM $ZnSO_4$ in their drinking water for a minimum of four days. On the day of the experiment the $ZnSO_4$ was relaced with normal drinking water and 60 min. later 30 - 60 uCi of $Na_2^{35}S$ was administered per os. The mice were sacrificed 60 min. later and intestinal tissue homogenised in a buffered aqueous solution containing 10mM dithiothreitol. Two volumes of performic acid were then added and the solution left at room temperature overnight. The suspension was then extracted with chloroform/methanol by conventional means and the aqueous layer concentrated by evaporation. Aliquots of the solution were then placed on Whatman 3mm filter paper and subjected to electrophoresis in a solution of pyridine:acetic acid:$H_2O$ (10:100:900, pH3.6) at a voltage of 200 Volts for 2 hr. The paper was the cut into 0.5 cm strips and radioactivity counted in a scintillation counter under standard conditions. The results are shown in Figure 8. As can be seen from these results the transgenic mice were able to synthesise radiolabelled cysteine from the administered sodium sulphide in contrast to the control mice.

TABLE 4

Expression of MTAceA2 and MTAceB2 in transformed mouse L-cells

| cell line | isocitrate lyase | malate synthase |
|---|---|---|
| control | 0 | 0 |
| MTAceA2 | 68 | - |
| MTAceB2 | - | 34.3 |

Values are nmoles product/mg protein/20 min

TABLE 5

Expression of MTAceAB1 in transgenic mice

| Mouse | Tissue | Isocitrate Lyase | Malate Synthase |
|---|---|---|---|
| control | intestine | not detectable | not detectable |
| | liver | not detectable | not detectable |
| | kidney | not detectable | not detectable |
| MTAceAB1 | intestine | 27.2 | ND |
| | liver | not detectable | 182 |
| | kidney | not detectable | 1.6 |

Values of isocitrate lyase are nmoles product/mg protein/20 min, and for malate synthase are picomoles product/mg protein/20 min (x $10^{-2}$)

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS:-

1. A genetic expression cassette for use in obtaining expression of a cDNA sequence in animal cells, the cassette comprising an inducible promoter and the 3' non-coding sequence of exon 5 of the growth hormone gene or a portion thereof, the cDNA sequence being positioned between the inducible promoter and the 3' non-coding sequence of exon 5 of the growth hormone gene.

2. A genetic expression cassette as claimed in claim 1 in which the inducible promoter is the immediate upstream nucleotide sequence of the sheep metallothionein-Ia gene.

3. A genetic expression cassette as claimed in claim 1 or claim 2 in which the cDNA codes for a bacterial enzyme, plant chitinase, insecticidal scorpion vermon toxin or the insecticidal protein of *Bacillus thuringiensis*.

4. A genetic expression cassette as claimed in claim 3 in which the cDNA sequence is selected from the group consisting of *cysE*, *cysK*, *aceA* and *aceB* genes of *Escherichia coli*.

5. A genetic expression cassette as claimed in claim 1 in which the expression cassette has a sequence substantially as shown in Figure 1.

6. A transgenic non-human animal including the genetic expression cassette as claimed in any one of claims 1 to 5.

7. A transgenic non-human animal as claimed in claim 6 in which the animal is ovine or bovine.

FIG. 1 1/2

SEQUENCE OF THE EXPRESSION CASSETTE

1 metallothionein promoter
gaattcaaagaggaaaagtgatgaaacaaggcttggcacagactccctggtatgtaattc
61
tcaggactattcaaagggaaatacccactgtcttacttcgttattggatgccagctctgc
121
ccatcacttacaaggatgcttttcctagggggcatcctatgactagggaacctccatcct
181
ggagccgggtggactggctaggcagtggattccctggcccattcatctattcagtcgtgg
241
agaatgtaaggaaggctgggcgacagaaggctgagttcgctgctgggctgttacaggaga
301
aactagagactctgttcaaagtccagggtgggggctgtgggaggaaatattagggaagcg
361
gggttcgggggataggtggtgaagctcacatccatcacgggtctctgcacacgacacagg
421
ggctccagccaagcctgggatgtgagcacgaggctcggattgcgcatgagctctgggaaa
481
gggtgaaagcaaagacaagagttgcgggggcagggaagactgcgaggactcagggactgg
541
gttcccgtaaacaccgatgactgcccacattgtggaaagctgggaaggggcgggcaggaa
601
tcctggagcgctacttgtcattcgggacaaagtccctccgcgttgggggcgagtaggggg
661
acggaggcgtttcggtgcgcacggagcccagccgcgttccgggaatcttgcgctcggccg
721
cgcgtggtgctcaccgcccgacccgggtgcagcgggcagctcgggtgcaggcgggggcag
781 metallothionein cap site *
accctctgcgcccggcccgcctcctgtgggtataatagcgctcggctcctgggctccaac
841
acgcctcccaccggaccagtggatccaca INSERT GENE IN THIS POSITION
910 growth hormone exon 5
tgtcctgtgatctaatgtcctgtgatcccgctgcgccttctagttgcca
960
gccatctgctgttacccctccctgtgccttcctagaccctggaaggtgccactccagtgc
1020
ccaccgtcctttcttaataaagcggaggaaattgcatcacattgtctgagtaggtgtcat
1080
tctattctaggggggtggggtcgggcaggatagcgagggggaggattgggaagacaatagc
1140
aggggtgctgtgggctctatgggtacccaggtgctgaataattgacccggttcctcctgg
1200
ggcagaaagaagcaggcacatccccttctctgtgacacacccggtcctcgcccctggtcc
1260
ttagttccagccccactcataggacactcacagctcaggagggctccgccttcaatccca
1320
cccgctaaagtgcttggagcggtctctccctctcagccaccagccgaatctaggcctcca

FIG. 1 2/2

1380
gagtgggaagaatttaagcaagacaggctatgaagtacagagggagagaaaatgcctcca
1440
acatgtgaggaagtgatgagagaaagcgtagaattagttttgtggcataaattttaaggt
1500
gactacacacttggcccaactacccttgggaaatgtgtgtgtgttagtcactcagttgtg
1560
tccagctctttgtgaccccacggactgtggctgccaggctcctctgtccatgggattctc
1620
cagggcaagaatactggaggggttgccattccccaggggatcttcccagcccaaggatc
1680
aaacccgagtttctgcattgcaggcagattctttactctctgagccatcagggaagccct
1740
gtgggaaatgggaaccatgcaagaatggctttgggaccaataggaccagaatgtttggga
1800
tctgaactgggtcaagagatgtggaagagagattctaaatgcatgtgttcatgctaagtg
1860
gcttcagtcgtgtcctactatttgcaaccccgatgaactgcagccaccaggctcctctgt
1920
catgggattctccattcaagaatactggagtgagtttccttcctccccaggggatctcca
1980
aacccagggattgaccaggatctcttgtatctcctggcacttgacaggcaaatctctcac
2040
cactagcgccactggacccagtctaag--unsequenced region

FIG. 2 1/3

SEQUENCE OF THE MTCE10 GENE

1 metallothionein promoter
gaattcaaagaggaaaagtgatgaaacaaggcttggcacagactccctggtatgtaattc
61
tcaggactattcaaagggaaatacccactgtcttacttcgttattggatgccagctctgc
121
ccatcacttacaaggatgcttttcctaggggcatcctatgactagggaacctccatcct
181
ggagccgggtggactggctaggcagtggattccctggcccattcatctattcagtcgtgg
241
agaatgtaaggaaggctgggcgacagaaggctgagttcgctgctgggctgttacaggaga
301
aactagagactctgttcaaagtccagggtggggctgtgggaggaaatattagggaagcg
361
gggttcggggataggtggtgaagctcacatccatcacgggtctctgcacacgacacagg
421
ggctccagccaagcctgggatgtgagcacgaggctcggattgcgcatgagctctgggaaa
481
gggtgaaagcaaagacaagagttgcgggggcagggaagactgcgaggactcagggactgg
541
gttcccgtaaacaccgatgactgcccacattgtggaaagctgggaaggggcgggcaggaa
601
tcctggagcgctacttgtcattcgggacaaagtccctccgcgttgggggcgagtagggg
661
acggaggcgtttcggtgcgcacggagcccagccgcgttccgggaatcttgcgctcggccg
721
cgcgtggtgctcaccgcccgacccgggtgcagcgggcagctcgggtgcaggcgggggcag
781 metallothionein cap site *
accctctgcgcccggcccgcctcctgtgggtataatagcgctcggctcctgggctccaac
841 bacterial <u>cysE</u> gene
MetSerCysGluGluLeuGluIleValTrpA
acgcctcccaccggaccagtggatccacaATGTCGTGTGAAGAACTGGAAATTGTCTGGA
901
snAsnIleLysAlaGluAlaArgThrLeuAlaAspCysGluProMetLeuAlaSerPheT
ACAATATTAAAGCCGAAGCCAGAACGCTGGCGGACTGTGAGCCAATGCTGGCCAGTTTTT
961
yrHisAlaThrLeuLeuLysHisGluAsnLeuGlySerAlaLeuSerTyrMetLeuAlaA
ACCACGCGACGCTACTCAAGCACGAAAACCTTGGCAGTGCACTGAGCTACATGCTGGCGA
1021
snLysLeuSerSerProIleMetProAlaIleAlaIleArgGluValValGluGluAlaT
ACAAGCTGTCATCGCCAATTATGCCTGCTATTGCTATCCGTGAAGTGGTGGAAGAAGCCT
1081
yrAlaAlaAspProGluMetIleAlaSerAlaAlaCysAspIleGlnAlaValArgThrA
ACGCCGCTGACCCGGAAATGATCGCCTCTGCGGCCTGTGATATTCAGGCGGTGCGTACCC
1141
rgAspProAlaValAspLysTyrSerThrProLeuLeuTyrLeuLysGlyPheHisAlaL
GCGACCCGGCAGTCGATAAATACTCAACCCCGTTGTTATACCTGAAGGGTTTTCATGCCT
1201
euGlnAlaTyrArgIleGlyHisTrpLeuTrpAsnGlnGlyArgArgAlaLeuAlaIleP
TGCAGGCCTATCGCATCGGTCACTGGTTGTGGAATCAGGGGCGTCGCGCACTGGCAATCT
1261
heLeuGlnAsnGlnValSerValThrPheGlnValAspIleHisProAlaAlaLysIleG

FIG. 2 2/3

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TTCTGCAAAACCAGGTTTCTGTGACGTTCCAGGTCGATATTCACCCGGCAGCAAAAATTG
1321
lyArgGlyIleMetLeuAspHisAlaThrGlyIleValValGlyGluThrAlaValIleG
GTCGCGGTATCATGCTTGACCACGCGACAGGCATCGTCGTTGGTGAAACGGCGGTGATTG
1381
luAsnAspValSerIleLeuGlnSerValThrLeuGlyGlyThrGlyLysSerGlyGlyA
AAAACGACGTATCGATTCTGCAATCTGTGACGCTTGGCGGTACGGGTAAATCTGGTGGTG
1441
spArgHisProLysIleArgGluGlyValMetIleGlyAlaGlyAlaLysIleLeuGlyA
ACCGTCACCCGAAAATTCGTGAAGGTGTGATGATTGGCGCGGGCGCGAAAATCCTCGGCA
1501
snIleGluValGlyArgGlyAlaLysIleGlyAlaGlySerValValLeuGlnProValP
ATATTGAAGTTGGGCGCGGCGCGAAGATTGGCGCAGGTTCCGTGGTGCTGCAACCGGTGC
1561
roProHisThrThrAlaAlaGlyValProAlaArgIleValGlyLysProAspSerAspL
CGCCGCATACCACCGCCGCTGGCGTTCCGGCTCGTATTGTCGGTAAACCAGACAGCGATA
1621
ysProSerMetAspMetAspGlnHisPheAsnGlyIleAsnHisThrPheGluTyrGlyA
AGCCATCAATGGATATGGACCAGCATTTCAACGGTATTAACCATACATTTGAGTATGGGG
1681
spGlyIle***           growth hormone exon 5
ATGGGATCTAAtgtcctgtgatctaatgtcctgtgatcccgctgcgccttctagttgcca
1741
gccatctgctgttacccctccctgtgccttcctagaccctggaaggtgccactccagtgc
1801
ccaccgtcctttcttaataaagcggaggaaattgcatcacattgtctgagtaggtgtcat
1861
tctattctaggggggtggggtcgggcaggatagcgaggggggaggattgggaagacaatagc
1921
aggggtgctgtgggctctatgggtacccaggtgctgaataattgacccggttcctcctgg
1981
ggcagaaagaagcaggcacatccccttctctgtgacacacccggtcctcgcccctggtcc
2041
ttagttccagccccactcataggacactcacagctcaggagggctccgccttcaatccca
2101
cccgctaaagtgcttggagcggtctctccctctcagccaccagccgaatctaggcctcca
2161
gagtgggaagaatttaagcaagacaggctatgaagtacagagggagagaaaatgcctcca
2221
acatgtgaggaagtgatgagagaaagcgtagaattagttttgtggcataaattttaaggt
2281
gactacacacttggcccaactacccttgggaaatgtgtgtgtgttagtcactcagttgtg
2341
tccagctctttgtgaccccacggactgtggctgccaggctcctctgtccatgggattctc
2401
cagggcaagaatactggaggggggttgccattccccaggggatcttcccagcccaaggatc
2461
aaacccgagtttctgcattgcaggcagattctttactctctgagccatcagggaagccct
2521
gtgggaaatgggaaccatgcaagaatggctttgggaccaataggaccagaatgtttggga
2581
tctgaactgggtcaagagatgtggaagagagattctaaatgcatgtgttcatgctaagtg
```

FIG. 2 3/3

2641
gcttcagtcgtgtcctactatttgcaaccccgatgaactgcagccaccaggctcctctgt
2701
catgggattctccattcaagaatactggagtgagtttccttcctccccaggggatctcca
2761
aacccagggattgaccaggatctcttgtatctcctggcacttgacaggcaaatctctcac
2821
cactagcgccactggacccagtctaag--unsequenced region

FIG. 3 1/3

SEQUENCE OF THE MTCK7 GENE

```
 1   metallothionein promoter
gaattcaaagaggaaaagtgatgaaacaaggcttggcacagactccctggtatgtaattc
 61
tcaggactattcaaagggaaatacccactgtcttacttcgttattggatgccagctctgc
 121
ccatcacttacaaggatgcttttcctagggggcatcctatgactagggaacctccatcct
 181
ggagccgggtggactggctaggcagtggattccctggcccattcatctattcagtcgtgg
 241
agaatgtaaggaaggctgggcgacagaaggctgagttcgctgctgggctgttacaggaga
 301
aactagagactctgttcaaagtccagggtgggggctgtgggaggaaatattagggaagcg
 361
gggttcggggggataggtggtgaagctcacatccatcacgggtctctgcacacgacacagg
 421
ggctccagccaagcctgggatgtgagcacgaggctcggattgcgcatgagctctgggaaa
 481
gggtgaaagcaaagacaagagttgcgggggcagggaagactgcgaggactcagggactgg
 541
gttcccgtaaacaccgatgactgcccacattgtggaaagctgggaaggggcgggcaggaa
 601
tcctggagcgctacttgtcattcgggacaaagtccctccgcgttgggggcgagtaggggg
 661
acggaggcgtttcggtgcgcacggagcccagccgcgttccgggaatcttgcgctcggccg
 721
cgcgtggtgctcaccgcccgacccgggtgcagcgggcagctcgggtgcaggcgggggcag
 781                                 metallothionein cap site  *
accctctgcgcccggcccgcctcctgtgggtataatagcgctcggctcctgggctccaac
 841                                    bacterial cysK gene
                                        MetSerLysIlePheGluAspAsnSer
acgcctcccaccggaccagtggatccgtcgaccATGAGTAAGATTTTTGAAGATAACTCG
 901
LeuThrIleGlyHisThrProLeuValArgLeuAsnArgIleGlyAsnGlyArgIleLeu
CTGACTATCGGTCACACGCCGCTGGTTCGCCTGAATCGCATCGGTAACGGACGCATTCTG
 961
AlaLysValGluSerArgAsnProSerPheSerValLysCysArgIleGlyAlaAsnMet
GCGAAGGTGGAATCTCGTAACCCCAGCTTCAGCGTTAAGTGCCGTATCGGTGCCAACATG
1021
IleTrpAspAlaGluLysArgGlyValLeuLysProGlyValGluLeuValGluProThr
ATTTGGGATGCCGAAAAGCGCGGCGTGCTGAAACCAGGCGTTGAACTGGTTGAACCGACC
1081
SerGlyAsnThrGlyIleAlaLeuAlaTyrValAlaAlaArgGlyTyrLysLeuThr
AGCGGTAATACCGGGATTGCACTGGCCTATGTAGCTGCCGCTCGCGGTTACAAACTCACC
1141
LeuThrMetProGluThrMetSerIleGluArgArgLysLeuLeuLysAlaLeuGlyAla
CTGACCATGCCAGAAACCATGAGTATTGAACGCCGCAAGCTGCTGAAAGCGTTAGGTGCA
1201
AsnLeuValLeuThrGluGlyAlaLysGlyMetLysGlyAlaIleGlnLysAlaGluGlu
AACCTGGTGCTGACGGAAGGTGCTAAAGGCATGAAAGGCGCAATCCAAAAAGCAGAAGAA
```

FIG. 3 2/3

```
1261
IleValAlaSerAsnProGluLysTyrLeuLeuLeuGlnGlnPheSerAsnProAlaAsn
ATTGTCGCCAGCAATCCAGAGAAATACCTGCTGCTGCAACAATTCAGCAATCCGGCAAAC
1321
ProGluIleHisGluLysThrThrGlyProGluIleTrpGluAspThrAspGlyGlnVal
CCTGAAATTCACGAAAAGACCACCGGTCCGGAGATATGGGAAGATACCGACGGTCAGGTT
1381
AspValPheIleAlaGlyValGlyThrGlyGlyThrTrpThrGlyValThrProTyrIle
GATGTATTTATTGCTGGCGTTGGGACTGGCGGTACGTGGACTGGCGTCACGCCCTACATT
1441
LysGlyThrLysGlyLysThrAspLeuIleSerValAlaValGluProThrAspSerPro
AAAGGCACCAAAGGCAAGACCGATCTTATCTCTGTCGCCGTTGAGCCAACCGATTCTCCA
1501
ValIleAlaGlnAlaLeuAlaGlyGluGluIleLysProGlyProHisLysIleGlnGly
GTTATCGCCCAGGCGCTGGCAGGTGAAGAGATTAAACCTGGCCCGCATAAAATTCAGGGT
1561
IleGlyAlaGlyPheIleProAlaAsnLeuAspLeuLysLeuValAspLysValIleGly
ATTGGCGCTGGTTTTATCCCGGCTAACCTCGATCTCAAGCTGGTCGATAAAGTCATTGGC
1621
IleThrAsnGluGluAlaIleSerThrAlaArgArgLeuMetGluGluGlyIleLeu
ATCACCAATGAAGAAGCGATTTCTACCGCGCGTCGTCTGATGGAAGAAGAAGGTATTCTT
1681
AlaGlyIleSerSerGlyAlaAlaValAlaAlaAlaLeuLysLeuGlnGluAspGluSer
GCAGGTATCTCTTCTGGAGCAGCTGTTGCCGCGGCGTTGAAACTACAAGAAGATGAAAGC
1741
PheThrAsnLysAsnIleValValIleLeuProSerSerGlyGluArgTyrLeuSerThr
TTTACCAACAAGAATATTGTGGTTATTCTACCATCATCGGGTGAGCGTTATTTAAGCACC
1801
AlaLeuPheAlaAspLeuPheThrGluLysGluLeuGlnGln*** growth hormone
GCATTGTTTGCCGATCTCTTCACTGAGAAAGAATTGCAACAGTAAtggccagctgcgcct
1861    exon 5
tctagttgccagccatctgctgttacccctccctgtgccttcctagaccctggaaggtgc
1921
cactccagtgcccaccgtcctttcttaataaagcggaggaaattgcatcacattgtctga
1981
gtaggtgtcattctattctaggggggtggggtcgggcaggatagcgagggggaggattggg
2041
aagacaatagcaggggtgctgtgggctctatgggtacccaggtgctgaataattgacccg
2101
gttcctcctggggcagaaagaagcaggcacatccccttctctgtgacacacccggtcctc
2161
gcccctggtccttagttccagccccactcataggacactcacagctcaggagggctccgc
2221
cttcaatcccacccgctaaagtgcttggagcggtctctccctctcagccaccagccgaat
2281
ctaggcctccagagtgggaagaatttaagcaagacaggctatgaagtacagagggagaga
2341
aaatgcctccaacatgtgaggaagtgatgagagaaagcgtagaattagttttgtggcata
2401
aattttaaggtgactacacacttggcccaactacccttgggaaatgtgtgtgtgttagtc
2461
actcagttgtgtccagctctttgtgaccccacggactgtggctgccaggctcctctgtcc
```

FIG. 3 3/3

2521
atgggattctccagggcaagaatactggaggggttgccattccccaggggatcttccca
2581
gcccaaggatcaaacccgagtttctgcattgcaggcagattctttactctctgagccatc
2641
agggaagccctgtgggaaatgggaaccatgcaagaatggctttgggaccaataggaccag
2701
aatgtttgggatctgaactgggtcaagagatgtggaagagagattctaaatgcatgtgtt
2761
catgctaagtggcttcagtcgtgtcctactatttgcaaccccgatgaactgcagccacca
2821
ggctcctctgtcatgggattctccattcaagaatactggagtgagtttccttcctcccca
2881
ggggatctccaaacccagggattgaccaggatctcttgtatctcctggcacttgacaggc
2941
aaatctctcaccactagcgccactggacccagtctaag---unsequenced region

FIG. 4 1/5

SEQUENCE OF THE MTCEK1 GENE

1 metallothionein promoter
atcatcgatcaggcagaattcaaagaggaaaagtgatgaaacaaggcttggcacagactc
61
cctggtatgtaattctcaggactattcaaagggaaatacccactgtcttacttcgttatt
121
ggatgccagctctgcccatcacttacaaggatgcttttcctagggggcatcctatgacta
181
gggaacctccatcctggagccgggtggactggctaggcagtggattccctggcccattca
241
tctattcagtcgtggagaatgtaaggaaggctgggcgacagaaggctgagttcgctgctg
301
ggctgttacaggagaaactagagactctgttcaaagtccagggtgggggctgtgggagga
361
aatattagggaagcggggttcggggggataggtggtgaagctcacatccatcacgggtctc
421
tgcacacgacacaggggctccagccaagcctgggatgtgagcacgaggctcggattgcgc
481
atgagctctgggaaagggtgaaagcaaagacaagagttgcgggggcagggaagactgcga
541
ggactcagggactgggttcccgtaaacaccgatgactgcccacattgtggaaagctggga
601
aggggcgggcaggaatcctggagcgctacttgtcattcgggacaaagtccctccgcgttg
661
ggggcgagtaggggggacggaggcgtttcggtgcgcacggagcccagccgcgttccgggaa
721
tcttgcgctcggccgcgcgtggtgctcaccgcccgacccgggtgcagcgggcagctcggg
781
tgcaggcgggggcagaccctctgcgcccggcccgcctcctgtgggtataatagcgctcgg
841 bacterial cysE
gene
* metallothionein cap site - MetSerCysGluGluL
ctcctgggctccaacacgcctcccaccggaccagtggatccacaATGTCGTGTGAAGAAC
901
euGluIleValTrpAsnAsnIleLysAlaGluAlaArgThrLeuAlaAspCysGluProM
TGGAAATTGTCTGGAACAATATTAAAGCCGAAGCCAGAACGCTGGCGGACTGTGAGCCAA
961
etLeuAlaSerPheTyrHisAlaThrLeuLeuLysHisGluAsnLeuGlySerAlaLeuS
TGCTGGCCAGTTTTTACCACGCGACGCTACTCAAGCACGAAAACCTTGGCAGTGCACTGA
1021
erTyrMetLeuAlaAsnLysLeuSerSerProIleMetProAlaIleAlaIleArgGluV
GCTACATGCTGGCGAACAAGCTGTCATCGCCAATTATGCCTGCTATTGCTATCCGTGAAG
1081
alValGluGluAlaTyrAlaAlaAspProGluMetIleAlaSerAlaAlaCysAspIleG
TGGTGGAAGAAGCCTACGCCGCTGACCCGGAAATGATCGCCTCTGCGGCCTGTGATATTC
1141
lnAlaValArgThrArgAspProAlaValAspLysTyrSerThrProLeuLeuTyrLeuL
AGGCGGTGCGTACCCGCGACCCGGCAGTCGATAAATACTCAACCCCGTTGTTATACCTGA
1201
ysGlyPheHisAlaLeuGlnAlaTyrArgIleGlyHisTrpLeuTrpAsnGlnGlyArgA
AGGGTTTTCATGCCTTGCAGGCCTATCGCATCGGTCACTGGTTGTGGAATCAGGGGCGTC

FIG. 4 2/5

1261
rgAlaLeuAlaIlePheLeuGlnAsnGlnValSerValThrPheGlnValAspIleHisP
GCGCACTGGCAATCTTTCTGCAAAACCAGGTTTCTGTGACGTTCCAGGTCGATATTCACC
1321
roAlaAlaLysIleGlyArgGlyIleMetLeuAspHisAlaThrGlyIleValValGlyG
CGGCAGCAAAAATTGGTCGCGGTATCATGCTTGACCACGCGACAGGCATCGTCGTTGGTG
1381
luThrAlaValIleGluAsnAspValSerIleLeuGlnSerValThrLeuGlyGlyThrG
AAACGGCGGTGATTGAAAACGACGTATCGATTCTGCAATCTGTGACGCTTGGCGGTACGG
1441
lyLysSerGlyGlyAspArgHisProLysIleArgGluGlyValMetIleGlyAlaGlyA
GTAAATCTGGTGGTGACCGTCACCCGAAAATTCGTGAAGGTGTGATGATTGGCGCGGGCG
1501
laLysIleLeuGlyAsnIleGluValGlyArgGlyAlaLysIleGlyAlaGlySerValV
CGAAAATCCTCGGCAATATTGAAGTTGGGCGCGGCGCGAAGATTGGCGCAGGTTCCGTGG
1561
alLeuGlnProValProProHisThrThrAlaAlaGlyValProAlaArgIleValGlyL
TGCTGCAACCGGTGCCGCCGCATACCACCGCCGCTGGCGTTCCGGCTCGTATTGTCGGTA
1621
ysProAspSerAspLysProSerMetAspMetAspGlnHisPheAsnGlyIleAsnHisT
AACCAGACAGCGATAAGCCATCAATGGATATGGACCAGCATTTCAACGGTATTAACCATA
1681
hrPheGluTyrGlyAspGlyIle*** growth hormone exon 5
CATTTGAGTATGGGGATGGGATCTAAtgtcctgtgatctaatgtcctgtgatcccgctgc
1741
gccttctagttgccagccatctgctgttacccctccctgtgccttcctagaccctggaag
1801
gtgccactccagtgcccaccgtcctttcttaataaagcggaggaaattgcatcacattgt
1861
ctgagtaggtgtcattctattctaggggggtggggtcgggcaggatagcgagggggaggat
1921
tgggaagacaatagcaggggtgctgtgggctctatgggtacccaggtgctgaataattga
1981
cccggttcctcctggggcagaaagaagcaggcacatccccttctctgtgacacacccggt
2041
cctcgcccctggtccttagttccagccccactcataggacactcacagctcaggagggct
2101
ccgccttcaatcccacccgctaaagtgcttggagcggtctctccctctcagccaccagcc
2161
gaatctaggcctccagagtgggaagaatttaagcaagacaggctatgaagtacagaggga
2221
gagaaaatgcctccaacatgtgaggaagtgatgagagaaagcgtagaattagttttgtgg
2281
cataaattttaaggtgactacacacttggcccaactacccttgggaaatgtgtgtgtgtt
2341
agtcactcagttgtgtccagctctttgtgaccccacggactgtggctgccaggctcctct
2401
gtccatgggattctccagggcaagaatactggaggggttgccattccccaggggatctt
2461
cccagcccaaggatcaaacccgagtttctgcattgcaggcagattctttactctctgagc
2521
catcagggaagccctgtgggaaatgggaaccatgcaagaatggctttgggaccaatagga

FIG. 4 3/5

```
2581
ccagaatgtttgggatctgaactgggtcaagagatgtggaagagagattctaaatgcatg
2641
tgttcatgctaagtggcttcagtcgtgtcctactatttgcaaccccgatgaactgcaggc
                             metallothionein promoter
2701
atgcaagcttcagatcatcgatgaattcaaagaggaaaagtgatgaaacaaggcttggca
2761
cagactccctggtatgtaattctcaggactattcaaagggaaatacccactgtcttactt
2821
cgttattggatgccagctctgcccatcacttacaaggatgcttttcctagggggcatcct
2881
atgactagggaacctccatcctggagccgggtggactggctaggcagtggattccctggc
2941
ccattcatctattcagtcgtggagaatgtaaggaaggctgggcgacagaaggctgagttc
3001
gctgctgggctgttacaggagaaactagagactctgttcaaagtccagggtgggggctgt
3061
gggaggaaatattagggaagcggggttcgggggataggtggtgaagctcacatccatcac
3121
gggtctctgcacacgacacaggggctccagccaagcctgggatgtgagcacgaggctcgg
3181
attgcgcatgagctctgggaaagggtgaaagcaaagacaagagttgcgggggcagggaag
3241
actgcgaggactcagggactgggttcccgtaaacaccgatgactgcccacattgtggaaa
3301
gctgggaaggggcgggcaggaatcctggagcgctacttgtcattcgggacaaagtccctc
3361
cgcgttgggggcgagtaggggggacggaggcgtttcggtgcgcacggagcccagccgcgtt
3421
ccgggaatcttgcgctcggccgcgcgtggtgctcaccgcccgacccgggtgcagcgggca
3481
gctcgggtgcaggcgggggcagaccctctgcgcccggcccgcctcctgtgggtataatag
                                                bacterial cysK gene
3541
                     *  metallothionein cap site               MetSe
cgctcggctcctgggctccaacacgcctcccaccggaccagtggatccgtcgaccATGAG
3601
rLysIlePheGluAspAsnSerLeuThrIleGlyHisThrProLeuValArgLeuAsnAr
TAAGATTTTTGAAGATAACTCGCTGACTATCGGTCACACGCCGCTGGTTCGCCTGAATCG
3661
gIleGlyAsnGlyArgIleLeuAlaLysValGluSerArgAsnProSerPheSerValLy
CATCGGTAACGGACGCATTCTGGCGAAGGTGGAATCTCGTAACCCCAGCTTCAGCGTTAA
3721
sCysArgIleGlyAlaAsnMetIleTrpAspAlaGluLysArgGlyValLeuLysProGl
GTGCCGTATCGGTGCCAACATGATTTGGGATGCCGAAAAGCGCGGCGTGCTGAAACCAGG
3781
yValGluLeuValGluProThrSerGlyAsnThrGlyIleAlaLeuAlaTyrValAlaAl
CGTTGAACTGGTTGAACCGACCAGCGGTAATACCGGGATTGCACTGGCCTATGTAGCTGC
3841
aAlaArgGlyTyrLysLeuThrLeuThrMetProGluThrMetSerIleGluArgArgLy
CGCTCGCGGTTACAAACTCACCCTGACCATGCCAGAAACCATGAGTATTGAACGCCGCAA
```

FIG. 4 4/5

```
3901
sLeuLeuLysAlaLeuGlyAlaAsnLeuValLeuThrGluGlyAlaLysGlyMetLysGl
GCTGCTGAAAGCGTTAGGTGCAAACCTGGTGCTGACGGAAGGTGCTAAAGGCATGAAAGG
3961
yAlaIleGlnLysAlaGluGluIleValAlaSerAsnProGluLysTyrLeuLeuLeuGl
CGCAATCCAAAAAGCAGAAGAAATTGTCGCCAGCAATCCAGAGAAATACCTGCTGCTGCA
4021
nGlnPheSerAsnProAlaAsnProGluIleHisGluLysThrThrGlyProGluIleTr
ACAATTCAGCAATCCGGCAAACCCTGAAATTCACGAAAAGACCACCGGTCCGGAGATATG
4081
pGluAspThrAspGlyGlnValAspValPheIleAlaGlyValGlyThrGlyGlyThrTr
GGAAGATACCGACGGTCAGGTTGATGTATTTATTGCTGGCGTTGGGACTGGCGGTACGTG
4141
pThrGlyValThrProTyrIleLysGlyThrLysGlyLysThrAspLeuIleSerValAl
GACTGGCGTCACGCCCTACATTAAAGGCACCAAAGGCAAGACCGATCTTATCTCTGTCGC
4201
aValGluProThrAspSerProValIleAlaGlnAlaLeuAlaGlyGluGluIleLysPr
CGTTGAGCCAACCGATTCTCCAGTTATCGCCCAGGCGCTGGCAGGTGAAGAGATTAAACC
4261
oGlyProHisLysIleGlnGlyIleGlyAlaGlyPheIleProAlaAsnLeuAspLeuLy
TGGCCCGCATAAAATTCAGGGTATTGGCGCTGGTTTTATCCCGGCTAACCTCGATCTCAA
4321
sLeuValAspLysValIleGlyIleThrAsnGluGluAlaIleSerThrAlaArgArgLe
GCTGGTCGATAAAGTCATTGGCATCACCAATGAAGAAGCGATTTCTACCGCGCGTCGTCT
4381
uMetGluGluGluGlyIleLeuAlaGlyIleSerSerGlyAlaAlaValAlaAlaAlaLe
GATGGAAGAAGAAGGTATTCTTGCAGGTATCTCTTCTGGAGCAGCTGTTGCCGCGGCGTT
4441
uLysLeuGlnGluAspGluSerPheThrAsnLysAsnIleValValIleLeuProSerSe
GAAACTACAAGAAGATGAAAGCTTTACCAACAAGAATATTGTGGTTATTCTACCATCATC
4501
rGlyGluArgTyrLeuSerThrAlaLeuPheAlaAspLeuPheThrGluLysGluLeuGl
GGGTGAGCGTTATTTAAGCACCGCATTGTTTGCCGATCTCTTCACTGAGAAAGAATTGCA
4561
nGln***      growth hormone exon 5
ACAGTAAtggccagctgcgccttctagttgccagccatctgctgttacccctcccctgtgc
4621
cttcctagaccctggaaggtgccactccagtgcccaccgtcctttcttaataaagcggag
4681
gaaattgcatcacattgtctgagtaggtgtcattctattctaggggggtggggtcgggcag
4741
gatagcgagggggaggattgggaagacaatagcaggggtgctgtgggctctatgggtacc
4801
caggtgctgaataattgacccggttcctcctggggcagaaagaagcaggcacatcccctt
4861
ctctgtgacacacccggtcctcgcccctggtccttagttccagccccactcataggacac
4921
tcacagctcaggagggctccgccttcaatcccacccgctaaagtgcttggagcggtctct
4981
ccctctcagccaccagccgaatctaggcctccagagtgggaagaatttaagcaagacagg
```

FIG. 4 5/5

5041
ctatgaagtacagagggagagaaaatgcctccaacatgtgaggaagtgatgagagaaagc
5101
gtagaattagttttgtggcataaattttaaggtgactacacacttggcccaactaccctt
5161
gggaaatgtgtgtgtgttagtcactcagttgtgtccagctctttgtgaccccacggactg
5221
tggctgccaggctcctctgtccatgggattctccagggcaagaatactggaggggttgc
5281
cattccccaggggatcttcccagcccaaggatcaaacccgagtttctgcattgcaggcag
5341
attctttactctctgagccatcagggaagccctgtgggaaatgggaaccatgcaagaatg
5401
gctttgggaccaataggaccagaatgtttgggatctgaactgggtcaagagatgtggaag
5461
agagattctaaatgcatgtgttcatgctaagtggcttcagtcgtgtcctactatttgcaa
5521
ccccgatgaactgcaggcatgcaagcttcagctgc

FIG. 5 1/3

SEQUENCE OF THE MTAceA2 GENE

1 metallothionein promoter
gaattcaaagaggaaaagtgatgaaacaaggcttggcacagactccctggtatgtaattc
61
tcaggactattcaaagggaaatacccactgtcttacttcgttattggatgccagctctgc
121
ccatcacttacaaggatgcttttcctaggggcatcctatgactagggaacctccatcct
181
ggagccgggtggactggctaggcagtggattccctggcccattcatctattcagtcgtgg
241
agaatgtaaggaaggctgggcgacagaaggctgagttcgctgctgggctgttacaggaga
301
aactagagactctgttcaaagtccagggtgggggctgtgggaggaaatattagggaagcg
361
gggttcgggggataggtggtgaagctcacatccatcacgggtctctgcacacgacacagg
421
ggctccagccaagcctgggatgtgagcacgaggctcggattgcgcatgagctctgggaaa
481
gggtgaaagcaaagacaagagttgcgggggcagggaagactgcgaggactcagggactgg
541
gttcccgtaaacaccgatgactgcccacattgtggaaagctgggaaggggcgggcaggaa
601
tcctggagcgctacttgtcattcgggacaaagtccctccgcgttgggggcgagtaggggg
661
acggaggcgtttcggtgcgcacggagcccagccgcgttccgggaatcttgcgctcggccg
721
cgcgtggtgctcaccgcccgacccgggtgcagcgggcagctcgggtgcaggcgggggcag
781 metallothionein cap site *
accctctgcgcccggcccgcctcctgtgggtataatagcgctcggctcctgggctccaac
841 bacterial <u>ace A</u> sequence
MetLysThrArgThrGlnG
acgcctcccaccggaccagtggatcctctagagtcatcaccATGAAAACCCGTACACAAC
901
lnIleGluGluLeuGlnLysGluTrpThrGlnProArgTrpGluGlyIleThrArgProT
AAATTGAAGAATTACAGAAAGAGTGGACTCAACCGCGTTGGGAAGGCATTACTCGCCCAT
961
yrSerAlaGluAspValValLysLeuArgGlySerValAsnProGluCysThrLeuAlaG
ACAGTGCGGAAGATGTGGTGAAATTACGCGGTTCAGTCAATCCTGAATGCACGCTGGCGC
1021
lnLeuGlyAlaAlaLysMetTrpArgLeuLeuHisGlyGluSerLysLysGlyTyrIleA
AACTGGGCGCAGCGAAAATGTGGCGTCTGCTGCACGGTGAGTCGAAAAAAGGCTACATCA
1081
snSerLeuGlyAlaLeuThrGlyGlyGlnAlaLeuGlnGlnAlaLysAlaGlyIleGluA
ACAGCCTCGGCGCACTGACTGGCGGTCAGGCGCTGCAACAGGCGAAAGCGGGTATTGAAG
1141
laValTyrLeuSerGlyTrpGlnValAlaAlaAspAlaAsnLeuAlaAlaSerMetTyrP
CAGTCTATCTGTCGGGATGGCAGGTAGCGGCGGACGCTAACCTGGCGGCCAGCATGTATC
1201
roAspGlnSerLeuTyrProAlaAsnSerValProAlaValValGluArgIleAsnAsnT
CGGATCAGTCGCTCTATCCGGCAAACTCGGTGCCAGCTGTGGTGGAGCGGATCAACAACA

FIG. 5 2/3

1261
hrPheArgArgAlaAspGlnIleGlnTrpSerAlaGlyIleGluProGlyAspProArgT
CCTTCCGTCGTGCCGATCAGATCCAATGGTCCGCGGGCATTGAGCCGGGCGATCCGCGCT
1321
yrValAspTyrPheLeuProIleValAlaAspAlaGluAlaGlyPheGlyGlyValLeuA
ATGTCGATTACTTCCTGCCGATCGTTGCCGATGCGGAAGCCGGTTTTGGCGGTGTCCTGA
1381
snAlaPheGluLeuMetLysAlaMetIleGluAlaGlyAlaAlaAlaValHisPheGluA
ATGCCTTTGAACTGATGAAAGCGATGATTGAAGCCGGTGCAGCGGCAGTTCACTTCGAAG
1441
spGlnLeuAlaSerValLysLysCysGlyHisMetGlyGlyLysValLeuValProThrG
ATCAGCTGGCGTCAGTGAAGAAATGCGGTCACATGGGCGGCAAAGTTTTAGTGCCAACTC
1501
lnGluAlaIleGlnLysLeuValAlaAlaArgLeuAlaAlaAspValThrGlyValProT
AGGAAGCTATTCAGAAACTGGTCGCGGCGCGTCTGGCAGCTGACGTGACGGGCGTTCCAA
1561
hrLeuLeuValAlaArgThrAspAlaAspAlaAlaAspLeuIleThrSerAspCysAspP
CCCTGCTGGTTGCCCGTACCGATGCTGATGCGGCGGATCTGATCACCTCCGATTGCGACC
1621
roTyrAspSerGluPheIleThrGlyGluArgThrSerGluGlyPhePheArgThrHisA
CGTATGACAGCGAATTTATTACCGGCGAGCGTACCAGTGAAGGCTTCTTCCGTACTCATG
1681
laGlyIleGluGlnAlaIleSerArgGlyLeuAlaTyrAlaProTyrAlaAspLeuValT
CGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCT
1741
rpCysGluThrSerThrProAspLeuGluLeuAlaArgArgPheAlaGlnAlaIleHisA
GGTGTGAAACCTCCACGCCGGATCTGGAACTGGCGCGTCGCTTTGCACAAGCTATCCACG
1801
laLysTyrProGlyLysLeuLeuAlaTyrAsnCysSerProSerPheAsnTrpGlnLysA
CGAAATATCCGGGCAAACTGCTGGCTTATAACTGCTCGCCGTCGTTCAACTGGCAGAAAA
1861
snLeuAspAspLysThrIleAlaSerPheGlnGlnGlnLeuSerAspMetGlyTyrLysP
ACCTCGACGACAAAACTATTGCCAGCTTCCAGCAGCAGCTGTCGGATATGGGCTACAAGT
1921
heGlnPheIleThrLeuAlaGlyIleHisSerMetTrpPheAsnMetPheAspLeuAlaA
TCCAGTTCATCACCCTGGCAGGTATCCACAGCATGTGGTTCAACATGTTTGACCTGGCAA
1981
snAlaTyrAlaGlnGlyGluGlyMetLysHisTyrValGluLysValGlnGlnProGluP
ACGCCTATGCCCAGGGCGAGGGTATGAAGCACTACGTTGAGAAAGTGCAGCAGCCGGAAT
2041
heAlaAlaAlaLysAspGlyTyrThrPheValSerHisGlnGlnGluValGlyThrGlyT
TTGCCGCCGCGAAAGATGGCTATACCTTCGTATCTCACCAGCAGGAAGTGGGTACAGGTT
2101
yrPheAspLysValThrThrIleIleGlnGlyGlyAspValPheSerHisArgAlaAspA
ACTTCGATAAAGTGACGACTATTATTCAGGGCGGCGACGTCTTCAGTCACCGCGCTGACC
2161 growth hormone exon 5
rgLeuHis***
GGCTCCACTGAagaatcgcagttctaatttgacctgcgccttctagttgccagccatctg
2221
ctgttacccctccctgtgccttcctagaccctggaaggtgccactccagtgcccaccgtc
2281
ctttcttaataaagcggaggaaattgcatcacattgtctgagtaggtgtcattctattct

FIG. 5 3/3

2341
agggggtggggtcgggcaggatagcgagggggaggattgggaagacaatagcaggggtgc
2401
tgtgggctctatgggtacccaggtgctgaataattgacccggttcctcctggggcagaaa
2461
gaagcaggcacatccccttctctgtgacacacccggtcctcgcccctggtccttagttcc
2521
agccccactcataggacactcacagctcaggagggctccgccttcaatcccacccgctaa
2581
agtgcttggagcggtctctccctctcagccaccagccgaatctaggcctccagagtggga
2641
agaatttaagcaagacaggctatgaagtacagagggagagaaaatgcctccaacatgtga
2701
ggaagtgatgagagaaagcgtagaattagttttgtggcataaattttaaggtgactacac
2761
acttggcccaactacccttgggaaatgtgtgtgtgttagtcactcagttgtgtccagctc
2821
tttgtgaccccacggactgtggctgccaggctcctctgtccatgggattctccagggcaa
2881
gaatactggaggggggttgccattccccaggggatcttcccagcccaaggatcaaacccga
2941
gtttctgcattgcaggcagattctttactctctgagccatcagggaagccctgtgggaaa
3001
tgggaaccatgcaagaatggctttgggaccaataggaccagaatgtttgggatctgaact
3061
gggtcaagagatgtggaagagagattctaaatgcatgtgttcatgctaagtggcttcagt
3121
cgtgtcctactatttgcaaccccgatgaactgcag

FIG. 6 1/3

SEQUENCE OF THE MTAceB2 GENE

```
   1 metallothionein promoter
gaattcaaagaggaaaagtgatgaaacaaggcttggcacagactccctggtatgtaattc
  61
tcaggactattcaaagggaaatacccactgtcttacttcgttattggatgccagctctgc
 121
ccatcacttacaaggatgcttttcctagggggcatcctatgactagggaacctccatcct
 181
ggagccgggtggactggctaggcagtggattccctggcccattcatctattcagtcgtgg
 241
agaatgtaaggaaggctgggcgacagaaggctgagttcgctgctgggctgttacaggaga
 301
aactagagactctgttcaaagtccagggtgggggctgtgggaggaaatattagggaagcg
 361
gggttcgggggataggtggtgaagctcacatccatcacgggtctctgcacacgacacagg
 421
ggctccagccaagcctgggatgtgagcacgaggctcggattgcgcatgagctctgggaaa
 481
gggtgaaagcaaagacaagagttgcgggggcagggaagactgcgaggactcagggactgg
 541
gttcccgtaaacaccgatgactgcccacattgtggaaagctgggaaggggcgggcaggaa
 601
tcctggagcgctacttgtcattcgggacaaagtccctccgcgttgggggcgagtaggggg
 661
acggaggcgtttcggtgcgcacggagcccagccgcgttccgggaatcttgcgctcggccg
 721
cgcgtggtgctcaccgcccgacccgggtgcagcgggcagctcgggtgcaggcgggggcag
 781                                metallothionein cap site  *
accctctgcgcccggcccgcctcctgtgggtataatagcgctcggctcctgggctccaac
 841              bacterial aceB sequence
                                                 MetThrGluGlnAlaThrT
acgcctcccaccggaccagtggatcctctagagtcatcaccATGACTGAACAGGCAACAA
 901
hrThrAspGluLeuAlaPheThrArgProTyrGlyGluGlnGluLysGlnIleLeuThrA
CAACCGATGAACTGGCTTTCACAAGGCCGTATGGCGAGCAGGAGAAGCAAATTCTTACTG
 961
laGluAlaValGluPheLeuThrGluLeuValThrHisPheThrProGlnArgAsnLysL
CCGAAGCGGTAGAATTTCTGACTGAGCTGGTGACGCATTTTACGCCACAACGCAATAAAC
1021
euLeuAlaAlaArgIleGlnGlnGlnGlnAspIleAspAsnGlyThrLeuProAspPheI
TTCTGGCAGCGCGCATTCAGCAGCAGCAAGATATTGATAACGGAACGTTGCCTGATTTTA
1081
leSerGluThrAlaSerIleArgAspAlaAspTrpLysIleArgGlyIleProAlaAspL
TTTCGGAAACAGCTTCCATTCGCGATGCTGATTGGAAAATTCGCGGGATTCCTGCGGACT
1141
euGluAspArgArgValGluIleThrGlyProValGluArgLysMetValIleAsnAlaL
TAGAAGACCGCCGCGTAGAGATAACTGGCCCGGTAGAGCGCAAGATGGTGATCAACGCGC
1201
euAsnAlaAsnValLysValPheMetAlaAspPheGluAspSerLeuAlaProAspTrpA
TCAACGCCAATGTGAAAGTCTTTATGGCCGATTTCGAAGATTCACTGGCACCAGACTGGA
```

FIG. 6 2/3

1261
snLysValIleAspGlyGlnIleAsnLeuArgAspAlaValAsnGlyThrIleSerTyrT
ACAAAGTGATCGACGGGCAAATTAACCTGCGTGATGCGGTTAACGGCACCATCAGTTACA
1321
hrAsnGluAlaGlyLysIleTyrGlnLeuLysProAsnProAlaValLeuIleCysArgV
CCAATGAAGCAGGCAAAATTTACCAGCTCAAGCCCAATCCAGCGGTTTTGATTTGTCGGG
1381
alArgGlyLeuHisLeuProGluLysHisValThrTrpArgGlyGluAlaIleProGlyS
TACGCGGTCTGCACTTGCCGGAAAAACATGTCACCTGGCGTGGTGAGGCAATCCCCGGCA
1441
erLeuPheAspPheAlaLeuTyrPhePheHisAsnTyrGlnAlaLeuLeuAlaLysGlyS
GCCTGTTTGATTTTGCGCTCTATTTCTTCCACAACTATCAGGCACTGTTGGCAAAGGGCA
1501
erGlyProTyrPheTyrLeuProLysThrGlnSerTrpGlnGluAlaAlaTrpTrpSerG
GTGGTCCCTATTTCTATCTGCCGAAAACCCAGTCCTGGCAGGAAGCGGCCTGGTGGAGCG
1561
luValPheSerTyrAlaGluAspArgPheAsnLeuProArgGlyThrIleLysAlaThrL
AAGTCTTCAGCTATGCAGAAGATCGCTTTAATCTGCCGCGCGGCACCATCAAGGCGACGT
1621
euLeuIleGluThrLeuProAlaValPheGlnMetAspGluIleLeuHisAlaLeuArgA
TGCTGATTGAAACGCTGCCCGCCGTGTTCCAGATGGATGAAATCCTTCACGCGCTGCGTG
1681
spHisIleValGlyLeuAsnCysGlyArgTrpAspTyrIlePheSerTyrIleLysThrL
ACCATATTGTTGGTCTGAACTGCGGTCGTTGGGATTACATCTTCAGCTATATCAAAACGT
1741
euLysAsnTyrProAspArgValLeuProAspArgGlnAlaValThrMetAspLysProP
TGAAAAACTATCCCGATCGCGTCCTGCCAGACAGACAGGCAGTGACGATGGATAAACCAT
1801
heLeuAsnAlaTyrSerArgLeuLeuIleLysThrCysHisLysArgGlyAlaPheAlaM
TCCTGAATGCTTACTCACGCCTGTTGATTAAAACCTGCCATAAACGCGGTGCTTTTGCGA
1861
etGlyGlyMetAlaAlaPheIleProSerLysAspGluGluHisAsnAsnGlnValLeuA
TGGGCGGCATGGCGGCGTTTATTCCGAGCAAAGATGAAGAGCACAATAACCAGGTGCTCA
1921
snLysValLysAlaAspLysSerLeuGluAlaAsnAsnGlyHisAspGlyThrTrpIleA
ACAAAGTAAAAGCGGATAAATCGCTGGAAGCCAATAACGGTCACGATGGCACATGGATCG
1981
laHisProGlyLeuAlaAspThrAlaMetAlaValPheAsnAspIleLeuGlySerArgL
CTCACCCAGGCCTTGCGGACACGGCAATGGCGGTATTCAACGACATTCTCGGCTCCCGTA
2041
ysAsnGlnLeuGluValMetArgGluGlnAspAlaProIleThrAlaAspGlnLeuLeuA
AAAATCAGCTTGAAGTGATGCGCGAACAAGACGCGCCGATTACTGCCGATCAGCTGCTGG
2101
laProCysAspGlyGluArgThrGluGluGlyMetArgAlaAsnIleArgValAlaValG
CACCTTGTGATGGTGAACGCACCGAAGAAGGTATGCGCGCCAACATTCGCGTGGCTGTGC
2161
lnTyrIleGluAlaTrpIleSerGlyAsnGlyCysValProIleTyrGlyLeuMetGluA
AGTACATCGAAGCGTGGATCTCTGGCAACGGCTGTGTGCCGATTTATGGCCTGATGGAAG
2221
spAlaAlaThrAlaGluIleSerArgThrSerIleTrpGlnTrpIleHisHisGlnLysT
ATGCGGCGACGGCTGAAATTTCCCGTACCTCGATCTGGCAGTGGATCCATCATCAAAAAA

FIG. 6 3/3

```
2281
hrLeuSerAsnGlyLysProValThrLysAlaLeuPheArgGlnMetLeuGlyGluGluM
CGTTGAGCAATGGCAAACCGGTGACCAAAGCCTTGTTCCGCCAGATGCTGGGCGAAGAGA
2341
etLysValIleAlaSerGluLeuGlyGluGluArgPheSerGlnGlyArgPheAspAspA
TGAAAGTCATTGCCAGCGAACTGGGCGAAGAACGTTTCTCCCAGGGGCGTTTTGACGATG
2401
laAlaArgLeuMetGluGlnIleThrThrSerAspGluLeuIleAspPheLeuThrLeuP
CCGCACGCTTGATGGAACAGATCACCACTTCCGATGAGTTAATTGATTTCCTGACCCTGC
2461                                   growth hormone exon 5
roGlyTyrArgLeuLeuAla***
CAGGCTACCGCCTGTTAGCGTAAtttgacctgcgccttctagttgccagccatctgctgt
2521
taccccctccctgtgccttcctagaccctggaaggtgccactccagtgcccaccgtccttt
2581
cttaataaagcggaggaaattgcatcacattgtctgagtaggtgtcattctattctaggg
2641
ggtggggtcgggcaggatagcgagggggaggattgggaagacaatagcaggggtgctgtg
2701
ggctctatgggtacccaggtgctgaataattgacccggttcctcctggggcagaaagaag
2761
caggcacatccccttctctgtgacacacccggtcctcgcccctggtccttagttccagcc
2821
ccactcataggacactcacagctcaggagggctccgccttcaatcccacccgctaaagtg
2881
cttggagcggtctctccctctcagccaccagccgaatctaggcctccagagtgggaagaa
2941
tttaagcaagacaggctatgaagtacagagggagagaaaatgcctccaacatgtgaggaa
3001
gtgatgagagaaagcgtagaattagttttgtggcataaattttaaggtgactacacactt
3061
ggcccaactacccttgggaaatgtgtgtgtgttagtcactcagttgtgtccagctctttg
3121
tgaccccacggactgtggctgccaggctcctctgtccatgggattctccagggcaagaat
3181
actggaggggttgccattccccaggggatcttcccagcccaaggatcaaacccgagttt
3241
ctgcattgcaggcagattctttactctctgagccatcagggaagccctgtgggaaatggg
3301
aaccatgcaagaatggctttgggaccaataggaccagaatgtttgggatctgaactgggt
3361
caagagatgtggaagagagattctaaatgcatgtgttcatgctaagtggcttcagtcgtg
3421
tcctactatttgcaaccccgatgaactgcag
```

FIG. 7 1/5

SEQUENCE OF THE MTAceAB1 GENE

```
 1 metallothionein promoter
gaattcaaagaggaaaagtgatgaaacaaggcttggcacagactccctggtatgtaattc
 61
tcaggactattcaaagggaaatacccactgtcttacttcgttattggatgccagctctgc
 121
ccatcacttacaaggatgcttttcctagggggcatcctatgactagggaacctccatcct
 181
ggagccgggtggactggctaggcagtggattccctggcccattcatctattcagtcgtgg
 241
agaatgtaaggaaggctgggcgacagaaggctgagttcgctgctgggctgttacaggaga
 301
aactagagactctgttcaaagtccagggtgggggctgtgggaggaaatattagggaagcg
 361
gggttcgggggataggtggtgaagctcacatccatcacgggtctctgcacacgacacagg
 421
ggctccagccaagcctgggatgtgagcacgaggctcggattgcgcatgagctctgggaaa
 481
gggtgaaagcaaagacaagagttgcgggggcagggaagactgcgaggactcagggactgg
 541
gttcccgtaaacaccgatgactgcccacattgtggaaagctgggaaggggcgggcaggaa
 601
tcctggagcgctacttgtcattcgggacaaagtccctccgcgttgggggcgagtaggggg
 661
acggaggcgtttcggtgcgcacggagcccagccgcgttccgggaatcttgcgctcggccg
 721
cgcgtggtgctcaccgcccgacccgggtgcagcgggcagctcgggtgcaggcgggggcag
 781                                  metallothionein cap site  *
accctctgcgcccggcccgcctcctgtgggtataatagcgctcggctcctgggctccaac
 841             bacterial aceA sequence
                                            MetLysThrArgThrGlnG
acgcctcccaccggaccagtggatcctctagagtcatcaccATGAAAACCCGTACACAAC
 901
lnIleGluGluLeuGlnLysGluTrpThrGlnProArgTrpGluGlyIleThrArgProT
AAATTGAAGAATTACAGAAAGAGTGGACTCAACCGCGTTGGGAAGGCATTACTCGCCCAT
 961
yrSerAlaGluAspValValLysLeuArgGlySerValAsnProGluCysThrLeuAlaG
ACAGTGCGGAAGATGTGGTGAAATTACGCGGTTCAGTCAATCCTGAATGCACGCTGGCGC
1021
lnLeuGlyAlaAlaLysMetTrpArgLeuLeuHisGlyGluSerLysLysGlyTyrIleA
AACTGGGCGCAGCGAAAATGTGGCGTCTGCTGCACGGTGAGTCGAAAAAAGGCTACATCA
1081
snSerLeuGlyAlaLeuThrGlyGlyGlnAlaLeuGlnGlnAlaLysAlaGlyIleGluA
ACAGCCTCGGCGCACTGACTGGCGGTCAGGCGCTGCAACAGGCGAAAGCGGGTATTGAAG
1141
laValTyrLeuSerGlyTrpGlnValAlaAlaAspAlaAsnLeuAlaAlaSerMetTyrP
CAGTCTATCTGTCGGGATGGCAGGTAGCGGCGGACGCTAACCTGGCGGCCAGCATGTATC
1201
roAspGlnSerLeuTyrProAlaAsnSerValProAlaValValGluArgIleAsnAsnT
CGGATCAGTCGCTCTATCCGGCAAACTCGGTGCCAGCTGTGGTGGAGCGGATCAACAACA
```

FIG. 7 2/5

1261
hrPheArgArgAlaAspGlnIleGlnTrpSerAlaGlyIleGluProGlyAspProArgT
CCTTCCGTCGTGCCGATCAGATCCAATGGTCCGCGGGCATTGAGCCGGGCGATCCGCGCT
1321
yrValAspTyrPheLeuProIleValAlaAspAlaGluAlaGlyPheGlyGlyValLeuA
ATGTCGATTACTTCCTGCCGATCGTTGCCGATGCGGAAGCCGGTTTTGGCGGTGTCCTGA
1381
snAlaPheGluLeuMetLysAlaMetIleGluAlaGlyAlaAlaAlaValHisPheGluA
ATGCCTTTGAACTGATGAAAGCGATGATTGAAGCCGGTGCAGCGGCAGTTCACTTCGAAG
1441
spGlnLeuAlaSerValLysLysCysGlyHisMetGlyGlyLysValLeuValProThrG
ATCAGCTGGCGTCAGTGAAGAAATGCGGTCACATGGGCGGCAAAGTTTTAGTGCCAACTC
1501
lnGluAlaIleGlnLysLeuValAlaAlaArgLeuAlaAlaAspValThrGlyValProT
AGGAAGCTATTCAGAAACTGGTCGCGGCGCGTCTGGCAGCTGACGTGACGGGCGTTCCAA
1561
hrLeuLeuValAlaArgThrAspAlaAspAlaAlaAspLeuIleThrSerAspCysAspP
CCCTGCTGGTTGCCCGTACCGATGCTGATGCGGCGGATCTGATCACCTCCGATTGCGACC
1621
roTyrAspSerGluPheIleThrGlyGluArgThrSerGluGlyPhePheArgThrHisA
CGTATGACAGCGAATTTATTACCGGCGAGCGTACCAGTGAAGGCTTCTTCCGTACTCATG
1681
laGlyIleGluGlnAlaIleSerArgGlyLeuAlaTyrAlaProTyrAlaAspLeuValT
CGGGCATTGAGCAAGCGATCAGCCGTGGCCTGGCGTATGCGCCATATGCTGACCTGGTCT
1741
rpCysGluThrSerThrProAspLeuGluLeuAlaArgArgPheAlaGlnAlaIleHisA
GGTGTGAAACCTCCACGCCGGATCTGGAACTGGCGCGTCGCTTTGCACAAGCTATCCACG
1801
laLysTyrProGlyLysLeuLeuAlaTyrAsnCysSerProSerPheAsnTrpGlnLysA
CGAAATATCCGGGCAAACTGCTGGCTTATAACTGCTCGCCGTCGTTCAACTGGCAGAAAA
1861
snLeuAspAspLysThrIleAlaSerPheGlnGlnGlnLeuSerAspMetGlyTyrLysP
ACCTCGACGACAAAACTATTGCCAGCTTCCAGCAGCAGCTGTCGGATATGGGCTACAAGT
1921
heGlnPheIleThrLeuAlaGlyIleHisSerMetTrpPheAsnMetPheAspLeuAlaA
TCCAGTTCATCACCCTGGCAGGTATCCACAGCATGTGGTTCAACATGTTTGACCTGGCAA
1981
snAlaTyrAlaGlnGlyGluGlyMetLysHisTyrValGluLysValGlnGlnProGluP
ACGCCTATGCCCAGGGCGAGGGTATGAAGCACTACGTTGAGAAAGTGCAGCAGCCGGAAT
2041
heAlaAlaAlaLysAspGlyTyrThrPheValSerHisGlnGlnGluValGlyThrGlyT
TTGCCGCCGCGAAAGATGGCTATACCTTCGTATCTCACCAGCAGGAAGTGGGTACAGGTT
2101
yrPheAspLysValThrThrIleIleGlnGlyGlyAspValPheSerHisArgAlaAspA
ACTTCGATAAAGTGACGACTATTATTCAGGGCGGCGACGTCTTCAGTCACCGCGCTGACC
2161 growth hormone exon 5
rgLeuHis***
GGCTCCACTGAagaatcgcagttctaatttgacctgcgccttctagttgccagccatctg
2221
ctgttacccctccctgtgccttcctagaccctggaaggtgccactccagtgcccaccgtc
2281
ctttcttaataaagcggaggaaattgcatcacattgtctgagtaggtgtcattctattct

FIG. 7 3/5

2341
agggggtggggtcgggcaggatagcgagggggaggattgggaagacaatagcaggggtgc
2401
tgtgggctctatgggtacccaggtgctgaataattgacccggttcctcctggggcagaaa
2461
gaagcaggcacatccccttctctgtgacacacccggtcctcgcccctggtccttagttcc
2521
agccccactcataggacactcacagctcaggagggctccgccttcaatcccacccgctaa
2581
agtgcttggagcggtctctccctctcagccaccagccgaatctaggcctccagagtggga
2641
agaatttaagcaagacaggctatgaagtacagagggagagaaaatgcctccaacatgtga
2701
ggaagtgatgagagaaagcgtagaattagttttgtggcataaattttaaggtgactacac
2761
acttggcccaactacccttgggaaatgtgtgtgtgttagtcactcagttgtgtccagctc
2821
tttgtgaccccacggactgtggctgccaggctcctctgtccatgggattctccagggcaa
2881
gaatactggaggggggttgccattccccaggggatcttcccagcccaaggatcaaacccga
2941
gtttctgcattgcaggcagattctttactctctgagccatcagggaagccctgtgggaaa
3001
tgggaaccatgcaagaatggctttgggaccaataggaccagaatgtttgggatctgaact
3061
gggtcaagagatgtggaagagagattctaaatgcatgtgttcatgctaagtggcttcagt
3121 metallothionein promoter
cgtgtcctactatttgcaaccccgatgaactgcaggaattcaaagaggaaaagtgatgaa
3181
acaaggcttggcacagactccctggtatgtaattctcaggactattcaaagggaaatacc
3241
cactgtcttacttcgttattggatgccagctctgcccatcacttacaaggatgcttttcc
3301
taggggggcatcctatgactagggaacctccatcctggagccgggtggactggctaggcag
3361
tggattccctggcccattcatctattcagtcgtggagaatgtaaggaaggctgggcgaca
3421
gaaggctgagttcgctgctgggctgttacaggagaaactagagactctgttcaaagtcca
3481
gggtgggggctgtgggaggaaatattagggaagcggggttcgggggataggtggtgaagc
3541
tcacatccatcacgggtctctgcacacgacacaggggctccagccaagcctggatgtga
3601
gcacgaggctcggattgcgcatgagctctgggaaagggtgaaagcaaagacaagagttgc
3661
gggggcagggaagactgcgaggactcagggactgggttcccgtaaacaccgatgactgcc
3721
cacattgtggaaagctgggaaggggcgggcaggaatcctggagcgctacttgtcattcgg
3781
gacaaagtccctccgcgttgggggcgagtagggggacggaggcgtttcggtgcgcacgga

FIG. 7 4/5

```
3841
gcccagccgcgttccgggaatcttgcgctcggccgcgcgtggtgctcaccgcccgacccg
3901
ggtgcagcgggcagctcgggtgcaggcgggggcagaccctctgcgcccggcccgcctcct
3961  metallothionein cap site   *
gtgggtataatagcgctcggctcctgggctccaacacgcctcccaccggaccagtggatc
4021                          bacterial aceB sequence
                     MetThrGluGlnAlaThrThrThrAspGluLeuAlaPheThrAr
ctctagagtcatcaccATGACTGAACAGGCAACAACAACCGATGAACTGGCTTTCACAAG
4081
gProTyrGlyGluGlnGluLysGlnIleLeuThrAlaGluAlaValGluPheLeuThrGl
GCCGTATGGCGAGCAGGAGAAGCAAATTCTTACTGCCGAAGCGGTAGAATTTCTGACTGA
4141
uLeuValThrHisPheThrProGlnArgAsnLysLeuLeuAlaAlaArgIleGlnGlnGl
GCTGGTGACGCATTTTACGCCACAACGCAATAAACTTCTGGCAGCGCGCATTCAGCAGCA
4201
nGlnAspIleAspAsnGlyThrLeuProAspPheIleSerGluThrAlaSerIleArgAs
GCAAGATATTGATAACGGAACGTTGCCTGATTTTATTTCGGAAACAGCTTCCATTCGCGA
4261
pAlaAspTrpLysIleArgGlyIleProAlaAspLeuGluAspArgArgValGluIleTh
TGCTGATTGGAAAATTCGCGGGATTCCTGCGGACTTAGAAGACCGCCGCGTAGAGATAAC
4321
rGlyProValGluArgLysMetValIleAsnAlaLeuAsnAlaAsnValLysValPheMe
TGGCCCGGTAGAGCGCAAGATGGTGATCAACGCGCTCAACGCCAATGTGAAAGTCTTTAT
4381
tAlaAspPheGluAspSerLeuAlaProAspTrpAsnLysValIleAspGlyGlnIleAs
GGCCGATTTCGAAGATTCACTGGCACCAGACTGGAACAAAGTGATCGACGGGCAAATTAA
4441
nLeuArgAspAlaValAsnGlyThrIleSerTyrThrAsnGluAlaGlyLysIleTyrGl
CCTGCGTGATGCGGTTAACGGCACCATCAGTTACACCAATGAAGCAGGCAAAATTTACCA
4501
nLeuLysProAsnProAlaValLeuIleCysArgValArgGlyLeuHisLeuProGluLy
GCTCAAGCCCAATCCAGCGGTTTTGATTTGTCGGGTACGCGGTCTGCACTTGCCGGAAAA
4561
sHisValThrTrpArgGlyGluAlaIleProGlySerLeuPheAspPheAlaLeuTyrPh
ACATGTCACCTGGCGTGGTGAGGCAATCCCCGGCAGCCTGTTTGATTTTGCGCTCTATTT
4621
ePheHisAsnTyrGlnAlaLeuLeuAlaLysGlySerGlyProTyrPheTyrLeuProLy
CTTCCACAACTATCAGGCACTGTTGGCAAAGGGCAGTGGTCCCTATTTCTATCTGCCGAA
4681
sThrGlnSerTrpGlnGluAlaAlaTrpTrpSerGluValPheSerTyrAlaGluAspAr
AACCCAGTCCTGGCAGGAAGCGGCCTGGTGGAGCGAAGTCTTCAGCTATGCAGAAGATCG
4741
gPheAsnLeuProArgGlyThrIleLysAlaThrLeuLeuIleGluThrLeuProAlaVa
CTTTAATCTGCCGCGCGGCACCATCAAGGCGACGTTGCTGATTGAAACGCTGCCCGCCGT
4801
lPheGlnMetAspGluIleLeuHisAlaLeuArgAspHisIleValGlyLeuAsnCysGl
GTTCCAGATGGATGAAATCCTTCACGCGCTGCGTGACCATATTGTTGGTCTGAACTGCGG
4861
yArgTrpAspTyrIlePheSerTyrIleLysThrLeuLysAsnTyrProAspArgValLe
TCGTTGGGATTACATCTTCAGCTATATCAAAACGTTGAAAAACTATCCCGATCGCGTCCT
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FIG. 7 5/5

4921
uProAspArgGlnAlaValThrMetAspLysProPheLeuAsnAlaTyrSerArgLeuLe
GCCAGACAGACAGGCAGTGACGATGGATAAACCATTCCTGAATGCTTACTCACGCCTGTT
4981
uIleLysThrCysHisLysArgGlyAlaPheAlaMetGlyGlyMetAlaAlaPheIlePr
GATTAAAACCTGCCATAAACGCGGTGCTTTTGCGATGGGCGGCATGGCGGCGTTTATTCC
5041
oSerLysAspGluGluHisAsnAsnGlnValLeuAsnLysValLysAlaAspLysSerLe
GAGCAAAGATGAAGAGCACAATAACCAGGTGCTCAACAAAGTAAAAGCGGATAAATCGCT
5101
uGluAlaAsnAsnGlyHisAspGlyThrTrpIleAlaHisProGlyLeuAlaAspThrAl
GGAAGCCAATAACGGTCACGATGGCACATGGATCGCTCACCCAGGCCTTGCGGACACGGC
5161
aMetAlaValPheAsnAspIleLeuGlySerArgLysAsnGlnLeuGluValMetArgGl
AATGGCGGTATTCAACGACATTCTCGGCTCCCGTAAAAATCAGCTTGAAGTGATGCGCGA
5221
uGlnAspAlaProIleThrAlaAspGlnLeuLeuAlaProCysAspGlyGluArgThrGl
ACAAGACGCGCCGATTACTGCCGATCAGCTGCTGGCACCTTGTGATGGTGAACGCACCGA
5281
uGluGlyMetArgAlaAsnIleArgValAlaValGlnTyrIleGluAlaTrpIleSerGl
AGAAGGTATGCGCGCCAACATTCGCGTGGCTGTGCAGTACATCGAAGCGTGGATCTCTGG
5341
yAsnGlyCysValProIleTyrGlyLeuMetGluAspAlaAlaThrAlaGluIleSerAr
CAACGGCTGTGTGCCGATTTATGGCCTGATGGAAGATGCGGCGACGGCTGAAATTTCCCG
5401
gThrSerIleTrpGlnTrpIleHisHisGlnLysThrLeuSerAsnGlyLysProValTh
TACCTCGATCTGGCAGTGGATCCATCATCAAAAAACGTTGAGCAATGGCAAACCGGTGAC
5461
rLysAlaLeuPheArgGlnMetLeuGlyGluGluMetLysValIleAlaSerGluLeuGl
CAAAGCCTTGTTCCGCCAGATGCTGGGCGAAGAGATGAAAGTCATTGCCAGCGAACTGGG
5521
yGluGluArgPheSerGlnGlyArgPheAspAspAlaAlaArgLeuMetGluGlnIleTh
CGAAGAACGTTTCTCCCAGGGGCGTTTTGACGATGCCGCACGCTTGATGGAACAGATCAC
5581
rThrSerAspGluLeuIleAspPheLeuThrLeuProGlyTyrArgLeuLeuAla***
CACTTCCGATGAGTTAATTGATTTCCTGACCCTGCCAGGCTACCGCCTGTTAGCGTAAtt
5641 growth hormone exon 5
tgacctgcgccttctagttgccagccatctgctgttacccctcccctgtgccttcctagac
5701
cctggaaggtgccactccagtgcccaccgtcctttcttaataaagcggaggaaattgcat
5761
cacattgtctgagtaggtgtcattctattctaggggggtggggtcgggcaggatagcgagg
5821
gggaggattgggaagacaatagcaggggtgctgtgggctctatgggtacccaggtgctga
5881
ataattgacccggttcctcctggggcagaaagaagcaggcacatccccttctctgtgaca
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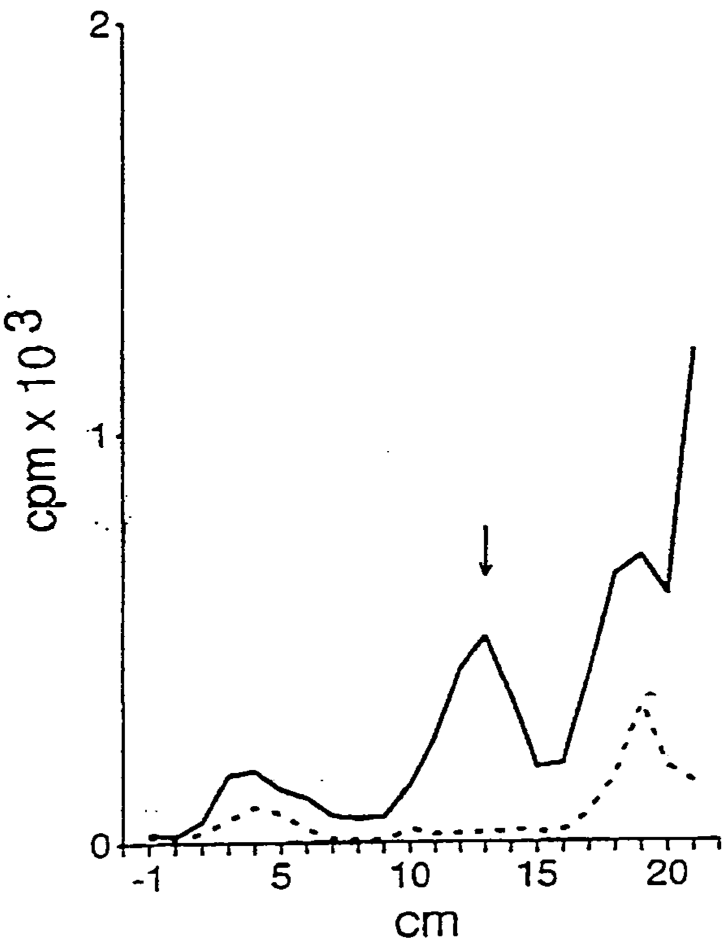


Fig. 8

Inte tional Application No. PCT/AU 92/00164

# INTERNATIONAL SEARCH REPORT

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent classification (IPC) or to both National Classification and IPC
Int. Cl.[5] C12N 15/85, 15/60, 15/67

## II. FIELDS SEARCHED

Minimum Documentation Searched [7]

| Classification System | Classification Symbols |
|---|---|
| IPC | WPAT Derwent Database: Keywords: inducible, promoter, regulatory, element, exon, non-coding<br>Chemical Abstracts: Keywords: hormone, exon, non-coding |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Biotechnology Abstracts: Keywords: growth, hormone, exon, non-coding
AU:IPC:C12N 15/85, 15/60, 15/67, 15/11, 15/18:

## III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

| Category* | Citation of Document, [11] with indication, where appropriate of the relevant passages [12] | Relevant to Claim No [13] |
|---|---|---|
| Y | Hampson, R.K. et al. Molecular and Cellular Biology, Volume 9, No. 4, April 1989 (American Society for Microbiology) "Alternative Processing of Bovine Growth Hormone mRNA is Influenced by Downstream Exon Sequences", see pages 1604-1610. | 1-7 |
| Y | Byrne, C.R. et al. Australian Journal of Biological Sciences, Volume 40, No. 4, 1987, "The Isolation and Characterisation of the Ovine Growth Hormone Gene", see pages 459-468. | 1-7 |
| Y | Orian, J.M. et al. Nucleic Acids Research, Volume 16, No. 18, 1988 (IRL Press Limited) "Cloning and sequencing of the ovine growth hormone gene" see page 9046. | 1-7 |

* Special categories of cited documents : [10]

"A" Document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior t the international filing date but later than the priority date claimed

"T" Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

## IV. CERTIFICATION

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 20 June 1992 | 25 June 1992 (25.06.92) |
| **International Searching Authority** | **Signature of Authorized Officer** |
| AUSTRALIAN PATENT OFFICE | M. ROSS |

**FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET**

| | | |
|---|---|---|
| A | Curatola, A.M. and C. Basilico. Molecular and Cellular Biology, Volume 10, No. 6, June 1980 (American Society for Microbiology) "Expression of the K-fgf Proto-Oncogene Is Controlled by 3[1] Regulatory Elements Which Are Specific for Embryonal Carcinoma Cells" see pages 2575-2483. | |
| A | Gutkind, J.S. et al. Molecular and Cellular Biology, Volume 11, No. 3, March 1991 (American Society for Microbiology) "A Novel c-fgr Exon Utilized in Epstein-Barr Virus-Infected B Lymphocytes but Not in Normal Monocytes" see pages 1500-1507. | |

**V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4a

**VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]**

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.